Posters

Protein Gymnastics

1271-Pos Board B1

Trypsinogen Activation Observed in Accelerated Molecular Dynamics Simulations

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Three decades ago Martin Karplus's group performed the first molecular dynamics simulations of trypsin in order to address the transition from the zymogen to its active form. Based on the low computational power existing at that time only high frequency fluctuations could be observed, but not the full transition, were observed. By performing accelerated molecular dynamics (aMD) simulations, a very promising approach to increase the configurational sampling of atomistic simulations, we were able to observe the amino tail insertion into the conserved hydrophobic pocket, a crucial step of the transition mechanism. The results allow us to address a very important enzymatic process, providing new evidence about the underlying thermodynamics.

1272-Pos Board B2

Conformational States and Dynamics of Neuronal Calcium Sensor Synaptotagmin

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Release of neuronal transmitters from nerve terminals is triggered by calcium binding to presynaptic protein synaptotagmin I (Syt). Syt is thought to interact with the SNARE protein complex, which mediates the attachment of synaptic vesicles to the plasma membrane. In addition, abundant evidence suggests that calcium binding promotes interaction of Syt with the plasma membrane, thus mediating synaptic vesicle fusion. Although calcium binding sites of Syt have been identified, it remains obscure how specifically calcium binding induces conformational changes in Syt and modifies its interactionswith the SNARE complex and presynaptic membrane to promote fusion. Syt is a vesicle transmembrane protein, and its soluble fragment includes two rigid domains connected by a flexible linker. Although the three-dimensional structure of Syt has been determined by crystallography, it has been demonstrated that multiple Syt conformations are likely to co-exist in the solution. Thus, it is still unclear what conformational states of Syt determine its function. To elucidate this question, we combined molecular dynamics and Monte-Carlo approaches to investigate Syt conformational states. We identified several conformational states of Syt, and we found that one of its low-energy conformations is characterized by a parallel arrangement of the domains in which all the calcium binding loops face the same surface. This conformation would facilitate Syt attachment to the plasma membrane and could potentially promote pore opening. We also found that calcium binding may promote Syt flexibility and facilitate transitions between conformational states.

1273-Pos Board B3

Active Output State of the Synechococcus Kai Circadian Oscillator

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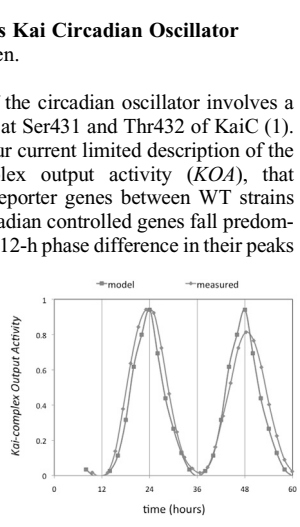
In cyanobacteria, the timekeeping aspect of the circadian oscillator involves a cyclic progression of phosphorylation states at Ser431 and Thr432 of KaiC (1). To identify oscillator signaling states with our current limited description of the output machinery, we defined Kai-complex output activity (*KOA*), that represents the difference in expression of reporter genes between WT strains and strains that lack KaiC (2). Although circadian controlled genes fall predominantly into one of two classes that differ in a 12-h phase difference in their peaks of expression, peak *KOA* for the two classes coincides late in the circadian cycle near subjective dawn, when KaiC-pST becomes most prevalent (Ser431 is phosphorylated and Thr432 is not). A mathematical expression involving a direct output from KaiC-pST and feedback inhibition by the output component RpaA using published data for the time-dependence of those states (3,4) successfully simulated two key features of the oscillator_the time of peak *KOA* and peak-to-trough amplitude (Figure).



(1) Mackey *et al* (2011) *Adv Genet.* 74, 13; (2) Paddock *et al* (2013) *Proc. Natl. Acad. Sci USA*, doi:10.1073/pnas.1315170110; (3) Gutu & O'Shea (2013) *Mol Cell* 50, 288; (4) Rust *et al* (2011) *Science* 318, 809.

1274-Pos Board B4

Determination of the Dynamic Structures of Igg Antibody by Individual-Particle Electron Tomography

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Antibody, also known as immunoglobulin, functions as one of the key parts in human immune response. Studying antibodies provides benefits in treating immune system diseases, medical diagnostics utilizing particular antibodies, and applications such as monoclonal antibody therapy. Antibodies perform functions through identifying and neutralizing various foreign objects based on a unique Y-shaped highly dynamic structure. In antibody structure, two antigen-binding domains (Fabs) are connected to effector domain (Fc) via a flexible hinge-loop region allowing two antigen-binding sites to vary. Dynamics of the loop region allow antibodies to undergo large conformational change to search binding sites on various biological macromolecule surfaces, fitting the paratope to a targeted antigen. Conventional techniques, such as X-ray crystallography, nuclear magnetic resonance technique, or single particle TEM reconstruction method, in studying antibody structure are faced with difficulty in revealing structural dynamic properties in 3D, due to their requirement of averaging vast numbers of particles.

Here, we use our recently developed individual-particle electron tomography (IPET) to obtain nearly a hundred 3D density maps each from an individual antibody. Each 3D map provided the spacing and orientations of the three domains of each antibody. By comparison of the domain spatial relation among different antibody molecules, the 3D distributions of the two F_{ab} domains uncovered the dynamics and fluctuations of this antibody type in solution. These dynamic characteristics provide us insight into antibody functional expression, and show potential approaches to increase antibody binding affinity.

1275-Pos Board B5

Pulsed EPR Distance Measurements Resolve the Impact of Site-Specific Calmodulin Methionine Oxidation

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We have examined the structural impact of oxidizing specific protein methionines (Met) in the C-ter lobe of calmodulin (CaM); these oxidation sites are known to abolish CaM regulation of the major calcium release channel, the ryanodine receptor complex (RyR). Protein oxidation by reactive oxygen species (ROS) is strongly associated with loss of strength in skeletal muscle and is proposed to play major roles in aging and degenerative muscle disease.

We have linked oxidation-induced changes in RyR regulation to changes in CaM-RyR structure using (1) protein mutagenesis to mimic oxidation at specific sites and (2) spectroscopy to resolve oxidation-induced changes in protein structural dynamics. Pulsed EPR distance measurements across CaM's lobes (multiple pairs of labeling sites, one label on each lobe) were sensitive to large-scale conformational changes that accompany both calcium binding and RyR peptide binding. In the absence of calcium, CaM was highly disordered, populating a broad distribution of conformations. Calcium binding strongly stabilized the elongated conformation, while RyR peptide binding to calcium-loaded CaM strongly stabilized the compact conformation. CaM conformation, particularly the distribution over structural states, was sensitive to Met to Gln substitutions (M109Q and M124Q) designed to mimic CaM Met oxidation. Structural sensitivity to M-to-Q mutations was observed in both the presence and absence of calcium, and in complex with RyR peptide. We conclude that Met oxidation alters CaM's functional interaction with RyR through changes in the orientation and flexibility of CaM's lobes.

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1276-Pos Board B6

Oxidative Stress Modulates Bioactivity of Lymphotoxin, but not TNF, Through Site-Specific Oxidation of Methionine Residues

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Lymphotoxin and TNF are a pair of highly homologous homotrimeric ligands that bind and activate TNF receptors 1 and 2. Activation of TNFR1 causes